Reproduction and larval growth of the urban dwelling Brown Striped Marsh Frog *Limnodynastes peronii*

Tanya J. Hengl and Shelley Burgin
Centre for Landscape and Ecosystems Management
University of Western Sydney
Locked Bag 1797 South Penrith Distribution Centre 1797
Corresponding author: A/Professor Shelley Burgin S.Burgin@uws.edu.au

ABSTRACT

Limnodynastes peronii was observed to successfully breed in small urban impoundments. Fecundity, egg viability and hatching success varied significantly, spatially and temporally. The predominant influence on breeding success was therefore deduced to be environmental. In contrast, growth varied more within populations than over time or between waterbodies and the observed results were considered to reflect genetic, rather than environmental, differences. Abnormalities were also hypothesised to reflect genetic differences and not environmental conditions.

Key words: *Limnodynastes peronii,* reproduction, larval growth, tadpole abnormalities.

Introduction

Generalised habitat descriptions for Brown striped marsh frogs *Limnodynastes peronii* differ among texts (eg., Cogger 2000; Griffiths 1997; Tyler 1992). For example, it has been recorded that it is found in lentic water, often inhabiting streams, creeks, marshes, dams and ponds and that they are also commonly found under debris in river flats (Cogger 2000; Griffiths 1997). Tyler (1992) defined their range as cool temperate to tropical, where they lived in swamps and open grasslands. Mahony (1993, 1996) recorded that they lived in permanent ponds, swamps and ephemeral pools. It has also been observed that the species is relatively rare in 'pristine', compared with degraded, areas (Ferraro and Burgin 1993; Schell 1997) and a number of authors (eg., Cogger 2000; Griffiths 1997; Voigt 1991) have recorded that they are inhabitants of garden ponds.

They have been shown to breed throughout the year after rainfall (Schell 1997), although like many other frogs they are less active during cooler periods (Griffiths 1997). The eggs are relatively easy to locate and identify, due to their foamy egg mass (Cogger 2000; Griffiths 1997; Tyler 1994). This 'nest' allows eggs to remain on the surface of the water and, in cooler climates, the foam can function to insulate the eggs and allow access to the warmest part of the water column (Tyler 1994). When the egg mass dries, its exterior turns tacky or hard and can therefore protect the enclosed embryoes from physical harm or predation (Duellman 1992).

Clutch sizes have been recorded to be between 705 and 1400 eggs/clutch, with egg diameter of approximately 1.5mm (Martin and Littlejohn 1982; Moore 1961; Schell 1997). Egg viability was observed to be between 64.1% - 83.4% (Schell 1997), while minimum age of metamorphosis has been recorded to be 11 - 12 days (Moore 1961). In our laboratory conditions (ambient temperature 19-25°C) larvae began to metamorphose after 12 weeks. It was also observed that there was an increase in fecundity as the season progressed and that tadpole growth rates were slower from egg masses deposited in winter than in summer (Schell 1997).

Larvae have been observed to thrive at pH 7 - 9, grew best at salinities of $\leq$7.5ppt and were more tolerant of 'high' salinity than *Uperoleia laevigata*. No mortality was observed in larvae maintained at 16 - 24°C (Ferraro 1992) and they grew more rapidly at lower density (Ferraro 1992; Voigt

1991). The effect of poor water quality (simulated agricultural pollution) was equivalent to growth of animals under crowded conditions: 5% of the weight increase of uncrowded animals in 'unpolluted' waters. However, larvae responded to changed environmental conditions: growth increased when conditions of crowding and pollution were alleviated (Voigt 1991). This was in contrast to the observations of Ferraro (1992) who observed that growth remained suppressed, despite death reducing density in some of his replicates.

Poor water quality can contribute to higher levels of abnormalities in disturbed environments (Sessions and Ruth 1990). Ferraro (1992; Ferraro and Burgin 1993) investigated non-traumatic and traumatic abnormality levels in disturbed and pristine environments in Western Sydney and observed that abnormalities in disturbed sites (12.5%) were significantly higher than undisturbed sites (2.5%).

Most of the limited data obtained on *L. peronii* has been from non-urban habitats, despite their apparent ability to breed in urban areas. In this paper we investigate reproductive success in such disturbance environments.

Methods

A range of sites in Western Sydney and the Blue Mountains was surveyed for *L. peronii* breeding activity (e.g. chorusing frogs and egg masses) between February and September 1998. Frogs were found at three urban sites (Blacktown, Pennant Hills and Faulconbridge) and egg masses from these sites were used as the basis of the study. There were no frog spore found at any of the other sites visited.

Study sites

The site located at Blacktown (Western Sydney) was on a creek within a local government park. The catchment was urban residential. The adjacent area was mowed, with a band of weed species, including water cress *Rorippa nasturtium-aquaticum*, buttercups *Ranunculus* sp. and curled dock *Rumex crispus* in the riparian zone. Overstorey vegetation was scattered eucalypts.

The waterbody investigated at Pennant Hills (north-west urban Sydney) was a small natural pond in an urban garden. The source of water was from a natural spring. No pesticides, herbicides or fertilizers were used on the surrounding gardens. Plants in the vicinity of the pond were exotic and adjacent to the pond ground cover was ivy and there were overhanging exotic shrubs.

The Faulconbridge site (lower Blue Mountains, west of Sydney) was an ornamental pond within an urban garden. The water level was maintained by rainwater. The site was located away from highways and no pesticides, insecticides or fertilizers had been used on the garden. The pond was overhung by tea-tree *Melaleuca* sp.

Egg collection and laboratory investigations

Searches were made after rainfall, and all egg masses observed were collected. Individual clutches were placed into a resealable plastic bag with 1L of water from the site. These were placed in a portable cooler to stabilise temperature and were transported to the laboratory within one hour of collection. The contents of the bags were then placed into individual 4L plastic containers and embryoes were left to develop and hatch for two to three days at ambient room temperature (20°C - 25°C). Upon hatching the number of live tadpoles and non-viable eggs were recorded for each egg mass. The live tadpoles were then placed in 4L plastic containers with 3L of tap water that had been left to age for 2 days (*cf.* aged tap-water).

To assess growth rate, three egg masses from each site, and collection time, were randomly selected from those used for clutch measurements. At two weeks of age, 20 tadpoles from each mass were randomly selected to monitor growth and to further investigate abnormalities. Each tadpole was placed in an individual plastic container (approximately 15 x 10 x 10 cm), containing 1L of aged water. Tadpoles were fed *ad libitum* with Nutrafin™ Goldfish Food throughout their captivity. Waste was removed every second day (with the aid of a plastic pipette or a fine net as appropriate), and there was a complete change of water every two weeks.

At monthly intervals each tadpole was blotted dry with paper towel and weighed (to 0.0001g). The growth trials were terminated after 18 weeks and tadpoles were released, after rain, at the point of collection.

In association with the assessment of reproductive variables, all larvae were examined for external abnormalities. To identify abnormalities that had escaped early detection, the 20 individuals from each egg mass used in the growth trials were monitored for abnormalities.

Differences in fecundity, egg viability and non-viable eggs were analysed using One-Way

ANOVA. Cochrans C test was used when heterogeneity of variances was detected. The heterogeneous variances were adjusted by using a log $(x+1)$ transformation. Ryans Q test was used to determine significant terms. To compare growth rates of tadpoles from three sites collected in March, the raw data were analysed using Two-Way Mixed Model ANOVA. While comparison of data collected from Blacktown and Faulconbridge was undertaken using a Three-Way Mixed Model ANOVA and homogeneity of variances was again tested, using Cochrans C test.

Results

Breeding activity was observed after two rainfall events (2-5 March, 12-27 April, 1998) at two sites (Faulconbridge and Blacktown) and once at Pennant Hills (2-5 March, 1998). There was no consistent trend among the sites or between sampling times. Fecundity varied within and among populations ($F=23.0$, $df_{1,4}$, $P<0.001$) (Figure 1). Analysis indicated that fecundity differed significantly among sites in March and was greatest at Blacktown and lowest at Pennant Hills (Table 1). Conversely in April, fecundity

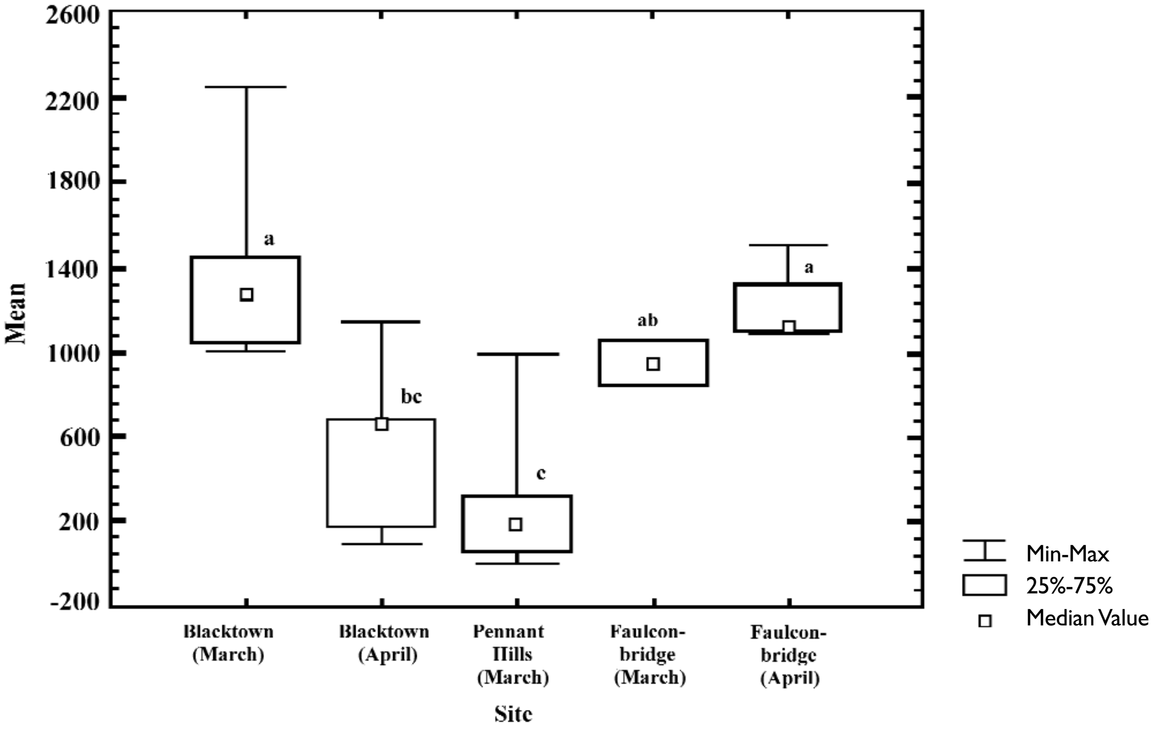

Figure 1. *Limnodynastes peronii* fecundity from three sites in Western Sydney investigated between March and April, 1998. Letters denote significance terms

Table 1. The number of viable and non-viable eggs/clutch from *Limnodynastes peronii* egg masses collected from three sites in Western Sydney between March and April, 1998 (± standard error).

		Site					
Egg Mass		Blacktown		Pennant Hills		Faulconbridge	
Parameters		March	April	March	April	March	April
Egg masses collected		5	5	23	0	2	4
Fecundity	Range	1011-2246	97-1149	3-1000	N/A	851-1064	1094-1516
	Mean	1407±225	555±192	238±46	N/A	N/A	1217±100
Viable eggs	Range	768-1831	95-1010	0-657	N/A	705-861	610-1436
	Mean	1132±203	455±170	172±33	N/A	N/A	960±189
Non-viable Eggs	Range	117-415	2-250	0-343	N/A	146-203	25-388
	Mean	275±50	99±44	60±18	N/A	N/A	176±80

was significantly higher at Faulconbridge than at Blacktown. A significant difference was also observed, between rainfall events, at one site (Blacktown). Although fecundity was lower from egg masses collected at this site later in the season, the trend was reversed, but not significantly different, at Faulconbridge.

Egg viability, measured as hatching success, differed among clutches within and between sites (F=19.0, $df_{1,4}$, P<0.001) (see Figure 2) and was highest at Blacktown in April (82.1%) and lowest, in March, at Pennant Hills (72.6%). Analyses revealed differences among sites in March and April. Within sites, egg viability differed between rainfall events. It was greater in March than April at Blacktown, but at Faulconbridge viability was lower from eggs collected after the second rainfall event.

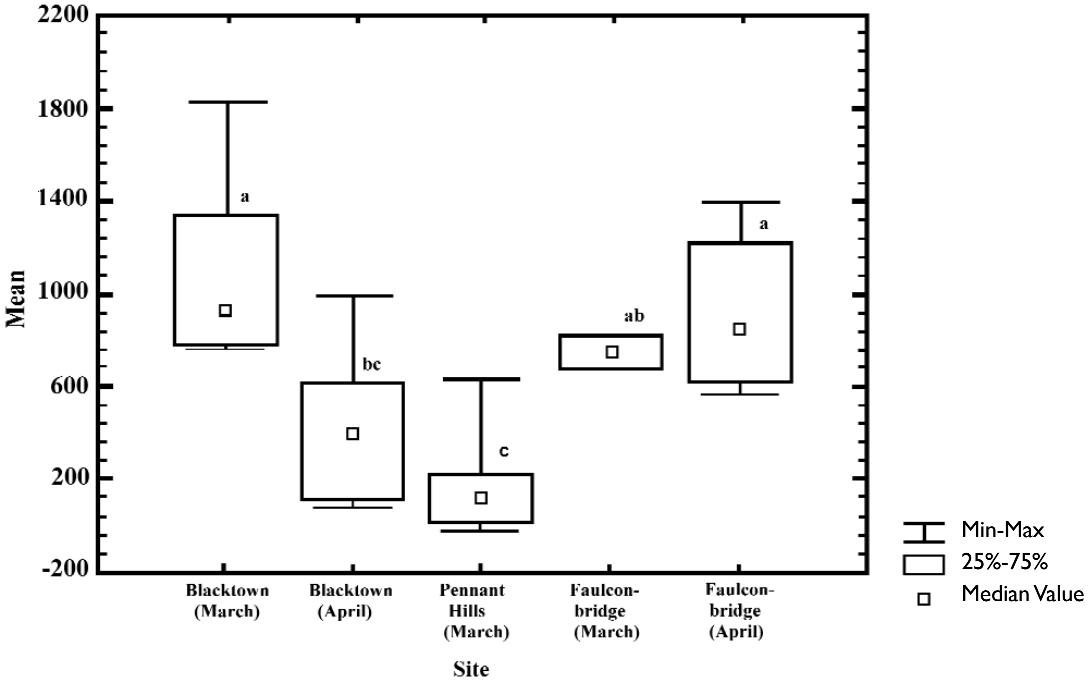

Figure 2: *Limnodynastes peronii* egg viability (hatching success) from three sites in Western Sydney investigated between March and April, 1998. Letters denote significance terms.

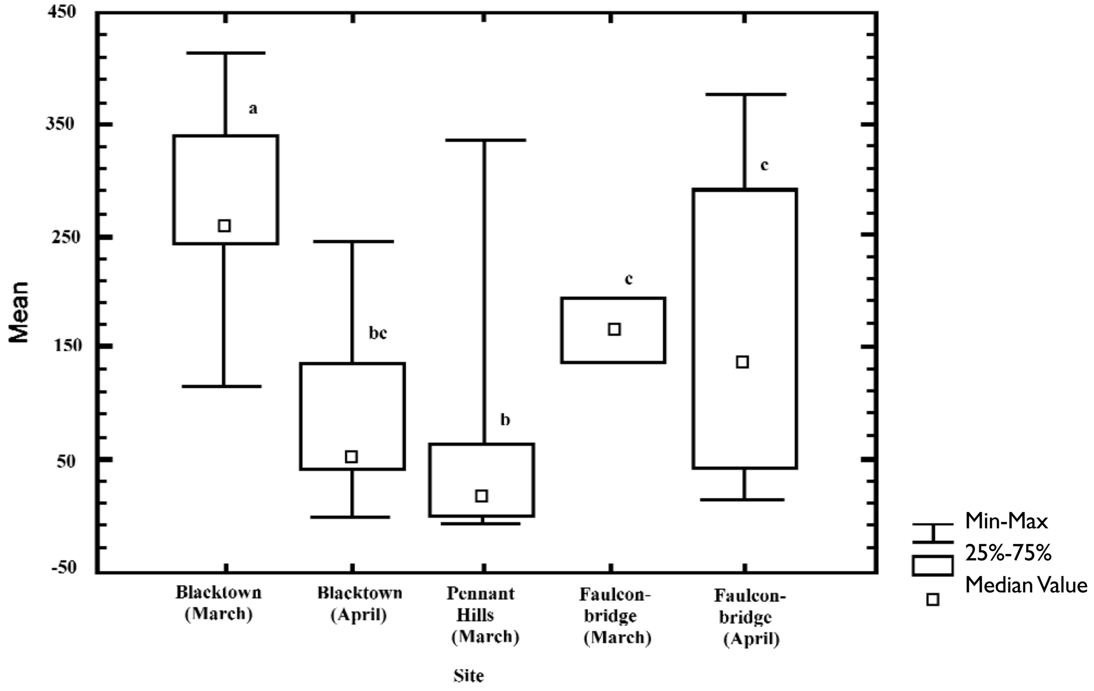

Figure 3: Non-viable *Limnodynastes peronii* eggs from three sites in Western Sydney, investigated between March and April, 1998. Letters denote significance terms.

The production of non-viable eggs also varied within and between sites (F=7.8, $df_{1,4}$, P=0.001) (Figure 3). Differences were again identified among sites in March. The greatest number of non-viable eggs was from the Pennant Hills population (21.4%), while the fewest non-viable eggs were recorded at Faulconbridge (17.8%). Conversely, in April, most non-viable eggs were observed at Faulconbridge (13.4%) and Blacktown had the lowest number (9.3%). In addition differences were observed between the two rainfall events at Blacktown but not at Faulconbridge.

Growth rates of tadpoles from eggs collected in March were observed to be similar among the three sites (Figure 4). Differences in growth rate were observed to be due to variation among egg masses (F=15.046, $df_{3,114}$, P<0.001) and not associated with site (F=0.336, $df_{2,114}$, P=0.715). Comparison of growth rates from eggs collected after different rainfall events indicated that differences were greater within clutches than between waterbodies (F=17.871, $df_{4,152}$, P<0.001). Rainfall event (F=1.092 $df_{1,1}$, P=0.486), site (F=0.264, $df_{1,4}$, P=0.635) and an interaction between these two effects (rainfall event and site; F=0.083, $df_{1,4}$, P=0.787) were not sufficient to significantly influence growth rates.

Abnormality levels were highest, and most variable, in the Pennant Hills population (Table 2) where deformities were observed in 67% (n=18) of the egg masses collected, compared with 14% (n=7) from Blacktown and 25% (n=4) from Faulconbridge. During the growth trials, an additional 10% of larvae (n=60) from Pennant Hills were observed to have anophthalmy (absence of eye/s).

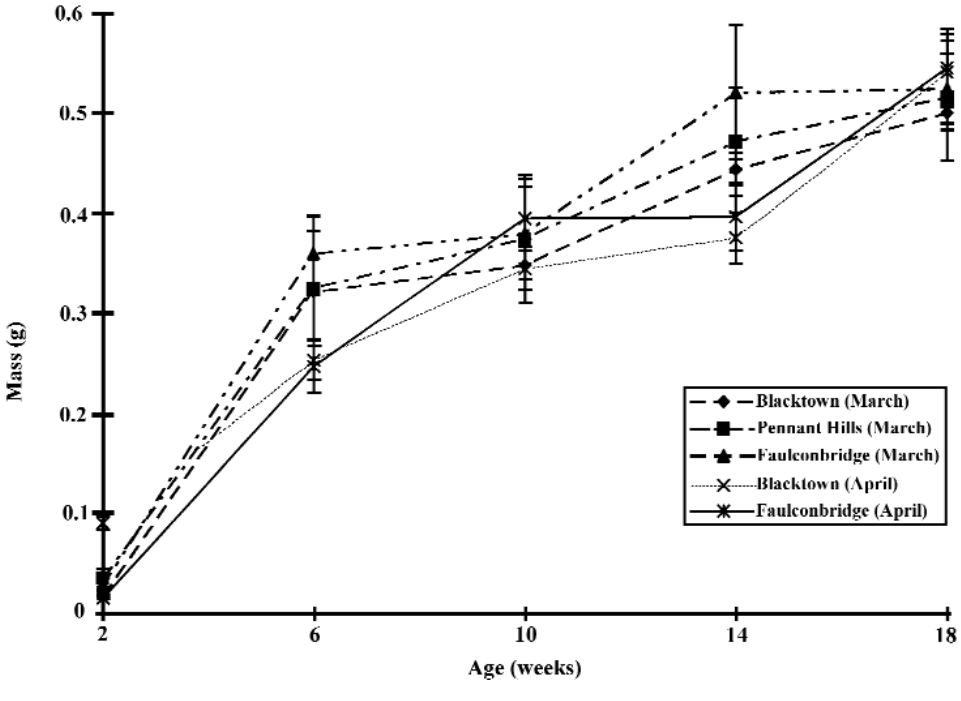

Figure 4. Average growth rate for *Limnodynastes peronii* tadpoles collected from three sites in Western Sydney during two collection times in March and April, 1998 (± standard error).

Table 2. Percent *Limnodynastes peronii* tadpole abnormalities observed during measurements of fecundity from egg masses collected in March and April, 1998 in Western Sydney.

Abnormality	Blacktown	Pennant Hills	Faulconbridge
Anophthalmy (absence of eye/s)	0	0.14%	0
Kinky tail	0.02%	0.34%	0
Abbreviation of tail	0.12%	0.14%	0
Body abnormality	0.07%	0.14%	0.02%
	n=3982	n=3479	n=3837

Discussion

These data confirm that small ornamental waterbodies in or adjacent to urban gardens are suitable for successful *L. peronii* reproduction, however, viability varies spatially and temporally. Parameters, such as fecundity and egg viability, are developmentally plastic and may be influenced by genetic and environmental factors. For example, reproduction within populations can be highly variable, due to factors such as temperature, rainfall or food availability, prior to vitellogenesis. We observed that fecundity varied among populations and with time and differed from previous records of clutch sizes (705-1009, Tyler 1994; 750-1400, Schell 1997). The observations of Schell (1997) were based on the Blacktown population and, in contrast to the results obtained in this study, he observed that fecundity increased as the season progressed (March - July). Since temperature may stimulate vitellogenic growth of ooyctes, higher clutch numbers may be produced in warmer weather (Kaplan 1987; Pancharatna and Patil 1997), and since trends were different between years, it is assumed that the differences were due to environmental factors.

Egg viability also differed significantly among sites and between rainfall events, in contrast to previous data collected from the Blacktown site (Schell 1997). Previously differences were not observed to be significant between rainfall events. These temporal and spatial differences are assumed to also be attributable to environmental differences.

Clutch parameters may vary across altitudinal and latitudinal gradients (Williamson and Bull 1995) and high elevation and low temperatures have been demonstrated to reduce clutch size (Cummins 1986; Williamson and Bull 1995). If altitude had an effect on *L. peronii*, this could have been reflected in differences between the Faulconbridge population and the two other, lower elevation, populations. This was not the case.

Larval growth differed among females but not sites. Unlike the other parameters investigated, these differences are hypothesised to be predominantly genetic. This is because variation in growth in the laboratory would not be differentially influenced by environmental factors, such as temperature, photoperiod and food availability (Travis 1981), since individuals were maintained under controlled environmental parameters.

Tadpole abnormality data for natural populations are limited but the level of abnormalities observed within the current study (0.2->10%) were within the range previously reported (natural populations 3%, Tyler 1994; 0-2.2%, Vershinin 1989; urban environments 1.5-15%, Vershinin 1989). The observation of eye abnormalities at one site could have been either environmental or genetic. Although untested, it has been suggested (Ferraro and Burgin 1993) that due to their greater abundance in disturbed areas than in pristine habitats, *L. peronii* was more resistant to urban pollutants than other local endemic species. However, the Pennant Hills site, where the greatest number of animals was observed with deformities, was least likely to have been recently exposed to pollutants due to gardening practices. The other major potential pollution source was vehicle emissions. Since only one site (Blacktown) could have been directly impacted from road runoff and all three could have been subjected to airborne vehicular pollutants, it is hypothesised that the eye deformities had a genetic basis.

In contrast to most reproductive characteristics investigated, larval growth did not differ between rainfall events or among sites and, therefore, this variation was considered to be due to genetic plasticity.

As indicated above, a range of factors has been presented as possible reasons for amphibian decline. Unlike many species currently under threat, *L. peronii* is able to take advantage of human-made or degraded sites unsuitable for other frog species. Some habitat degradation may therefore advantage, rather than disadvantage this species. Variability in growth under laboratory conditions indicated a genetic plasticity that would allow some resistance to environmental fluctuation. However, since most clutch characteristics were apparently influenced by environment, climate change may ultimately have an adverse impact on the species despite their ability to take advantage of human modification of their immediate environment.

Acknowledgments

We would like to thank Chris Schell for his support and useful comments throughout this study and to Dr Charles Morris for his advice on statistical analyses.

References

Cogger, H.G. 2000. *Reptiles and Amphibians of Australia*. 6th Edition. Reed Books, Sydney.

Cummins, C.P. 1986. Temporal and spatial variation in egg size and fecundity in *Rana temporaria*. *Journal of Animal Ecology* **55**: 303-316.

Duellman, W.E. 1992. Reproductive strategies of frogs. *Scientific American.* July 1992.

Ferraro, T.J. 1992. Factors influencing the decline of frogs (Amphibia: Anura) in Western Sydney. B.Ap.Sc.(Hons), University of Western Sydney - Hawkesbury, Richmond.

Ferraro, T.J. and Burgin, S. 1993. Amphibian decline: a case study in Western Sydney. Pp 197-204 in *Herpetology in Australia: A Diverse Discipline*, edited by D. Lunney and D. Ayers. Transactions of the Royal Zoological Society of New South Wales, Mosman.

Griffiths, K. 1997. *Frogs and Reptiles of the Sydney Region*. Bush Books, University of New South Wales Press, Sydney.

Kaplan, R.H. 1987. Developmental plasticity and maternal effects of reproductive characteristics in the frog, *Bombina orientalis*. *Oecologia* **71**: 273-279.

Mahony, M.J. 1993. The status of frogs in the Watagan Mountains area, the Central Coast of New South Wales. Pp 257-264 in *Herpetology in Australia: A Diverse Discipline*, edited by D. Lunney and D. Ayers. Transactions of the Royal Zoological Society of New South Wales, Mosman.

Mahony, M.J. 1996. The decline of the Green and Golden Bell Frog *Litoria aurea* viewed in the context of declines and disappearances of other Australian frogs. *Australian Zoologist* **30**: 237-247.

Martin A.A. and Litttlejohn, M.J. 1982. *Tasmanian Amphibians*. Fauna of Tasmania Handbook No 6. University of Tasmania, Hobart.

Moore, J.A. 1961. Frogs of eastern New South Wales. *Bulletin of the American Museum of Natural History* **121**: 149-386.

Pancharatna, K. and Patil, M.M. 1997. Role of temperature and photoperiod in the onset of sexual maturity in female frogs, *Rana cyanophlyctis*. *Journal of Herpetology* **31**: 111-114.

Schell, C.B. 1997. Effects of time and habitat status of frog (Amphibia:Anura) reproduction: a case study from Western Sydney. BSc (Hons) University of Western Sydney - Hawkesbury, Richmond.

Sessions, S.K. and Ruth, S.B. 1990. Explanation for naturally occurring supernumerary limbs in amphibians. *Journal of Experimental Zoology* **254**: 38-47.

Travis, J. 1981. Control of larval growth variation in a population of *Pseudacris triseriata* (Anura:Hylidae). *Evolution* **35**: 423-432.

Tyler, M.J. 1992. Encyclopedia of Australian animals. *The national photographic index of Australian wildlife. Frogs*. Angus and Robertson, Sydney.

Tyler, M.J. 1994. *Australian Frogs: A Natural History*. Reed Books, Chatswood.

Vershinin, V.L. 1989. Morphological anomalies in urban amphibians. *Ekologiya* (Sverdlovsk, Russia) 3:58-66, cited in Ouellet M., Bonin J., Rodrigue J., DesGranges J-L. and Lair S. 1997. Hindlimb deformities (Ectromelia, Ectrodactyly) in free-living anurans from agricultural habitats. *Journal of Wildlife Diseases* **33**(1): 95-104.

Voigt, M. 1991. On the effects of crowding and pollution in tadpoles of the Brown Striped Marsh Frog (*Limnodynastes peronii*). *Herpetofauna* **21**: 25-34.

Williamson, I. and Bull, C.M. 1995. Life-history in a population of the Australian frog *Ranidella signifera: seasonal changes in clutch parameters*. *Copeia* **1995**: 105-115.